REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the instant application are respectfully requested in view of the amendments to the claims and remarks presented herewith, which place the application into condition for allowance.

Status of the Claims and Formal Matters

Claims 1-35 are currently pending in this application. Of these, claims 9-11 and 27-35 were withdrawn from consideration as allegedly being drawn to a non-elected invention and a non-elected species. Applicants assert the right to reclaim withdrawn or cancelled subject matter in co-pending applications. By this paper, Claims 1, 16, 17, 19, and 21 are amended, without prejudice, and solely to expedite prosecution pursuant to the U.S. Patent and Trademark Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). Claims 8 and 22 have been cancelled. No new matter has been introduced by these amendments. Support for the amended recitations can be found throughout the specification as originally filed, particularly at page 16, lines 7-23 of the application published as WO04/029294, and original claims 8 and 22.

Rejections under 35 U.S.C. §112, 1st paragraph

Claims 19 and 21 were rejected under 35 U.S.C. §112, 1st paragraph as allegedly failing to comply with the written description requirement. The Office Action contends that the claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had possession of the claimed invention. In particular, the Office Action contends that these claims allegedly recite that the primer comprises dATP or ATP that is exchanged for the alpha-S-analog "from the beginning (rather than as a result of the primer extension process)." See Office Action at page 3. According to the Office Action, there is allegedly no support in the original disclosure for such subject matter.

Applicants respectfully disagree and contend that these claims and the requisite support in the specification adequately describe to those skilled in the art that the alpha-S-analog is incorporated by way of primer extension, however in the interest of expediting prosecution,

Claims 19 and 21 have been amended to further clarify that the alpha-S-analog is incorporated into the extended RNA or DNA primer, thereby obviating the rejection. Applicants respectfully request reconsideration and withdrawal of the §112, 1st paragraph rejection.

Rejections under 35 U.S.C. §103(a)

Claims 1-8, 12-18, 22, 23, 25, and 26 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Nyren et al (U.S. Patent No. 6,210,891; hereinafter "Nyren") in view of Melamede (U.S. Patent No. 4,863,849; "Melamede"), Kotewicz et al (U.S. Patent No. 5,244,797; "Kotewicz"), and Inouye et al (U.S. Patent No. 5,434,070; "Inouye"). According to the Office Action, Nyren allegedly discloses a method of determining the sequence of a nucleic acid molecule, but does not disclose that the nucleic acid being sequenced is RNA, that the method is performed in the presence of an RNase-inhibiting agent, or that the polymerase is a reverse transcriptase essentially lacking RNase H activity. Nyren also does not teach a reverse transcriptase as recited in instant Claim 12, or a mixture of RNA-dependent polymerases. Nyren is also deficient for failing to disclose performing the extension at a temperature within the range recited in instant Claim 14, or a nucleotide concentration within the range recited in Claim 16. Nyren also fails to teach any of the compounds recited in instant Claim 22.

Melamede allegedly teaches a sequencing method by annealing a primer to a DNA or RNA template, performing a primer extension reaction, and detecting incorporation. According to the Office Action, Melamede also allegedly teaches AMV reverse transcriptase. Although Melamede does not disclose mixtures of RNA dependent polymerases or amplification of the RNA molecule prior to sequencing, the Office Action contends that the mixtures of RNA dependent polymerases allegedly represent an obvious variation that combines known equivalents for the same purpose. The Office Action further contends that it would have been obvious to amplify the RNA before sequencing since Nyren allegedly taught it was desirable to amplify the nucleic acid to be sequenced in situations where the amount of sample available was small. Melamede, notably, does not disclose reverse transcriptases that lack RNase H activity.

Kotewicz allegedly teaches a reverse transcriptase that essentially lacks RNase H activity and also allegedly discloses use of the enzyme to perform primer extension from an RNA template at a nucleotide concentration of 0.5 mM, at a salt concentration of 75 mM, and in the presence of actinomycin D. Kotewicz also allegedly discloses that this enzyme is used to perform primer extension from an RNA template using a DNA primer. According to the Office Action, it would allegedly have been *prima facie* obvious to one of ordinary skill in the art to use the RNase deficient reverse transcriptase as allegedly taught by Kotewicz for the purpose of sequencing an RNA molecule by the method suggested by the combined teachings of Nyren and Melamede. The Office Action further argues that it would also allegedly have been obvious to use the conditions of temperature, pH, salt, nucleotide, and actinomycin D concentrations, since Kotewicz allegedly showed these were appropriate conditions.

Inouye allegedly teaches the inclusion of RNase inhibitors in the reverse transcription reaction mixture, such as, for example, vanadyl-ribonucleoside complexes or RNasin. The Office Action contends that the art is replete with examples of the inclusion of RNase inhibitors in reverse transcription reactions. Further, according to the Office Action, it would allegedly have been *prima facie* obvious to one of ordinary skill in the art to use an RNase inhibitor when practicing the RNA sequencing method suggested by the combined teachings of Nyren, Melamede, and Kotewicz, since it was allegedly known in the art that RNA was susceptible to RNase degradation and Inouye allegedly teaches that it was known in the art to add inhibitors of RNase to a reverse transcription reaction.

In view of the amendments to the claims and remarks presented herewith, Applicants respectfully traverse this rejection.

As the Office Action concedes, Nyren does not disclose methods of determining the identity of at least one nucleotide in an RNA molecule, nor does Nyren disclose the use of an RNA-dependent polymerase, such as a reverse transcriptase essentially lacking RNase H activity. Nyren is also silent regarding an extension reaction solution comprising, *inter alia*, a RNA-secondary structure reducing reagent as claimed in Claim 1 (and claims dependent therefrom).

Melamede relates to a method for determining the nucleotide sequence of DNA or RNA molecules. Like Nyren, Melamede also fails to disclose an extension reaction solution comprising reagents to detect light triggered by the release of PPi and RNA secondary structure reducing reagents as in instant Claim 1.

Kotewicz describes genes encoding reverse transcriptase having DNA polymerase activity and substantially no RNase H activity, as well as vectors containing the gene, hosts transformed with vectors containing the gene, and methods of producing cDNA from mRNA using the reverse transcriptase. Notably, Kotewicz is silent as to an amplification reaction solution comprising reagents to detect light triggered by the release of PPi and RNA-secondary structure reducing reagents as claimed in instant Claim 1.

Inouye discloses multi-copy single-stranded DNA/RNA hybrid structures termed "msDNAs." Inouye also describes reverse transcriptases that are capable of synthesizing a cDNA molecule from an RNA template that forms a 2',5'-linkage between the template molecule and the first nucleotide at the 5' end of the cDNA strand. Like Nyren, Melamede, and Kotewicz, Inouye fails to disclose extension reaction solutions comprising reagents to detect light triggered by the release of PPi and RNA secondary structure reducing reagents as recited in instant Claim 1.

Whether considered individually or in combination with each other, these references do not establish *prima facie* obviousness under §103(a) because these references fail to teach or disclose all of the instant claim limitations. None of Nyren, Melamede, Kotewicz, or Inouye disclose RNA secondary structure reducing reagents. In view of these deficiencies, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection over Nyren in view of Melamede, Kotewicz, and Inouye.

Claim 20 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Nyren in view of Melamede, Kotewicz, and Inouye, and further in view of Myers et al (Proc. Natl. Acad. Sci. 77(3): 1316-1310 (1980); "Myers"). None of Nyren, Melamede, Kotewicz, or Inouye teach the use of an RNA primer, however Myers allegedly teaches that reverse

transcriptases can uses RNA as a primer. The Office Action contends that it would allegedly have been *prima facie* obvious to one of ordinary skill in the art to use either DNA or RNA as the primer when practicing the method for sequencing RNA suggested by the combined teachings of Nyren, Melamede, Kotewicz, and Inouye, since "these are the only two options" and both were known in the art to be suitable for primer extension by reverse transcriptase. Applicants respectfully traverse.

As discussed herein, none of Nyren, Melamede, Kotewicz, or Inouye teach or disclose all of the instant claim limitations, namely an extension reaction solution comprising, *inter alia*, reagents to detect light triggered by the release of PPi and RNA secondary structure reducing reagents as recited in instant Claim 1 (from which Claim 20 depends). Myers does not cure this deficiency. Myers describes studies involving avian myeloblastosis virus (AMV) reverse transcriptase and its ability to create fragments of the original RNA templates (by virtue of its RNase H activity) that act as primers for the synthesis of anticomplementary DNA. Myers is silent regarding extension reaction solutions comprising RNA secondary structure reducing reagents as set forth in the instant claims. Because the combination of Nyren, Melamede, Kotewicz, Inouye, and Myers do not teach or disclose all of the instant claim limitations, Applicants respectfully contend that *prima facie* obviousness has not been established. The rejection should be withdrawn.

Claim 24 was rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Nyren in view of Melamede, Kotewicz, and Inouye and further in view of Malek et al (U.S. Patent No. 5,665,545; "Malek"). Nyren, Melamede, Kotewicz, and Inouye do not teach using rITP in place of rGTP during amplification of the RNA, however Malek teaches a method of amplifying RNA called "terminal repeat amplification method" (TRAM) and allegedly teaches that substitution of rITP for rGTP in an RNA amplification produce alleviates pausing of reverse transcriptase due to secondary structure (stem-loop formation) when using the RNA in a subsequent primer extension reaction. The Office Action further contends that it would allegedly have been *prima facie* obvious to the skilled artisan to use the known method of RNA amplification as allegedly taught by Malek when practicing the method for sequencing RNA

allegedly suggested by the combined teachings of Nyren, Melamede, Kotewicz, and Inouye. Applicants traverse.

As discussed elsewhere in this response, Nyren, Melamede, Kotewicz, and Inouye, considered separately or in combination with each other, do not teach or disclose all of the instant claim limitations, in particular, extension reaction solutions comprising reagents to detect light triggered by the release of PPi and RNA secondary structure reducing reagents as set forth in instant Claim 1 (from which Claim 24 indirectly depends). Malek is also silent regarding this claim recitation. Malek relates to methods of amplifying a specific nucleic acid sequence or its complement at constant temperatures and without serial addition of reagents. The Malek method teaches that an RNA first template is used in the reaction medium, which comprises an inverted repeat sequence which folds into a 5' terminal stem-loop structure, which is then used by DNA polymerase in the reaction medium to synthesize an RNA-DNA hybrid molecule, ultimately resulting in a partially double-stranded DNA having a promoter oriented toward the apex of the stem-loop structure. See, for example, Malek, abstract and col. 4, lines 5-17. Thus, the Malek method relies on the presence of the stem-loop structure to achieve the desired amplification of the specific nucleic acid sequence or its complement and inclusion of an RNA secondary structure reducing reagent would destroy or greatly minimize such stem-loop structures. Indeed, Malek is silent regarding RNA secondary structure reducing reagents as recited in the instant claims.

In view of the foregoing remarks, Applicants respectfully contend that *prima facie* obviousness under §103(a) has not been established, because the cited references do not teach or disclose all of the instant claim limitations. Reconsideration and withdrawal of the §103(a) rejection over Nyren, Melamede, Kotewicz, Inouye, and Malek are respectfully requested.

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion regarding this Response is desired, the Examiner is respectfully urged to contact the undersigned at the number given below, and is assured of full cooperation in progressing the application to allowance.

Applicants believe no additional fees are due with the filing of this Response. However, if any additional fees are required or if any funds are due, the USPTO is authorized to charge or credit Deposit Account Number: **50-0311**, Customer Number: **35437**, Reference Number: **21465-523 NATL**.

Respectfully submitted,

Dated: February 12, 2008

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